PATENT
File No.: 00-79D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Hart, Charles E. et al.
Serial No.	:	10/606,055
Filed	:	June 25. 2003
For	:	METHOD OF TREATING FIBROPROLIFERATIVE DISORDERS
Examiner	:	Borgeest, Christina M.
Art Unit	:	1649
Docket No.	:	00-79D1
Date	:	March 30, 2006

Commissioner for Patents
P.O. Box P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Debra G. Gilbertson Under 37 C.F.R. § 1.132

Sir:

I, Debra G. Gilbertson, do hereby declare as follows:

1. I am currently employed by ZymoGenetics, Inc., the assignee of the above-named patent application, as a Senior Scientist.

2. I received a Master of Science degree in Microbiology from North Dakota State University in 1990.

3. I am an inventor of the above-identified patent application ("the Patent Application").

4. I have read the Office Action mailed January 3, 2006 in the Patent Application, including the rejection under 35 U.S.C. § 112.

5. I have personal knowledge of the experiments described herein. Some of these experiments were published in the Journal of the American Society of Nephrology (Hudkins et al., "Exogenous PDGF-D Is a Potent Mesangial Cell Mitogen and Causes a Severe Mesangial Proliferative Glomerulopathy" *J Am Soc Nephrol* 15:286-298, 2004; Taneda et al., "Obstructive Uropathy in Mice and Humans: Potential Role for PDGF-D in the Progression of Tubulointerstitial Injury" *J Am Soc Nephrol* 14:2544-2555, 2003). I am a coauthor of these articles.

6. Zvegf4 (also known as PDGF-D), a new member of the PDGF family, is most like PDGF-B in its receptor-binding characteristics. Zvegf4, like PDGF-B, binds and signals through PDGF receptor β (PDGF-Rβ). Prior to the filing date of the Patent Application, it was known that members of the PDGF family (inlcuding PDGF-B) are potent inducers of mesangial cell proliferation and extracellular matrix production. Increased expression of PDGFs in renal disease has been well documented in the scientific literature. In addition, PDGF-B antagonism was known to result in decreased mesangial proliferation and matrix reduction in the Thy 1.1 mesangioproliferative nephritis model (an animal model of glomerulonephritis).

7. Experiments were undertaken to investigate the *in vitro* activity of zvegf4 on renal cells that play a major role in disease development. Mitogenic activity was assessed by the ability to stimulate incorporation of [^{3}H]thymidine into normal human mesangial cells. Zvegf4 was found to significantly induce mesangial cell proliferation at a concentration of 0.01 ng/ml. This effect was dose-dependent and plateaued at about 10 ng/ml with a sevenfold increase in tritium incorporation. The effects of zvegf4 on mesangial cells were as strong as those of PDGF-B, which (as noted above) has been shown to play a pivotal role in the mediation of glomerular mesangial proliferation. These experiments demonstrated a potent and direct mitogenic effect for zvegf4 on kidney mesangial cells, a key cell type involved in kidney (glomerular) fibrosis. Additional experiments demonstrated that the *in vitro* mitogenic activity of zvegf4 on various mesenchymal cell types could be neutralized by zvegf4-specific monoclonal antibodies.

8. Experiments were also undertaken to investigate effects of zvegf4 overexpression *in vivo* using a renal injury animal model. Adenovirus constructs encoding zvegf4 and other PDGF isoforms were injected into mice. After three weeks, the mice were sacrificed, and the kidneys were collected for analysis. A second study involved a time course of sacrifice at 2-week intervals (2, 4, 6, and 8 week time points). Tissue samples were analyzed by immunohistochemistry, TUNEL staining, morphometry, and electron

microscopy. Blood samples were analyzed for PDGF levels (by ELISA) and blood chemistry. Control mice (adenovirus alone) demonstrated no obvious histopathologic abnormalities. Mice injected with an adenoviral construct encoding zvegf4 had moderate to severe glomerulopathy, which was characterized as enlargement of the glomeruli and an increase in cellularity due to mesangial proliferation in the glomerular tuft. There was also a marked increase in extracellular matrix accumulation and increased macrophage counts. Animals infected with a PDGF-C adenoviral construct showed no evidence of renal pathology. Animals infected with a PDGF-B adenoviral construct had slight to moderate glomerulopathy in the kidney. As discussed above, PDGF-B and its receptors have already been shown to play a role in kidney fibrosis. These experiments demonstrated that renal injury (manifested as mesangial proliferative glomerulopathy) resulted from overexpression of zvegf4 in these animals.

9. We also investigated the expression of zvegf4 in kidney disease. In one study, residual paraffin-embedded, formalin-fixed renal nephrectomy tissues from ten patients with chronic obstructive nephropathy were studied for expression of zvegf4, PDGF-B, PDGF-Rβ, alpha smooth muscle actin, and type I and type IV collagens by immunohistochemistry. In these specimens, there was persistent expression of PDGF-D by glomerular visceral epithelial cells and vascular smooth muscle cells, as well as *de novo* expression by periglomerular interstitial cells and by some neointimal cells of arteriosclerotic vessels. These observations suggest that zvegf4 plays an important role in the pathogenesis of tubulointerstitial injury through binding of PDGF-Rβ in human obstructive nephropathy. In a second study, expression of zvegf4 and PDGF-Rβ in human kidneys was studied using immunohistochemistry, RT-PCR, and immunostaining. Zvegf4 was found to be expressed by visceral epithelial cells (podocytes) in mature human kidneys. Zvegf4 was also strongly expressed by medial smooth muscle cells of arteries and arterioles in mature and developing human kidneys. It was persistently expressed by medial smooth muscle cells as well as some neointimal smooth muscle cells in arteriosclerosis, acute and chronic vascular rejection, and cyclosporin A / FK-506 arteriopathy. Zvegf4 was also identified at the basolateral membrane of some injured tubules in areas of tubulinterstitial injury. PDGF-Rβ (the receptor for zvegf4) was expressed by mesangial cells, interstitial cells, and vascular smooth muscle cells, suggesting potential autocrine and paracrine interactions via zvegf4 between these cells and visceral epithelial cells. These experiments show that zvegf4 has regulated expression in human kidney disease.

10. The following points summarize zvegf4's role in kidney disease. (a) Zvegf4 and its receptor are upregulated in fibroproliferative human kidney diseases. (b)

Zvegf4 is a potent inducer of mesangial cell proliferation both *in vitro* and *in vivo*. (c) *In vivo* overexpression of zvegf4 initiates events leading to mesangial proliferative glomerulonephritis. These data provide support for a causal role of zvegf4 in the pathogenesis of fibroproliferative kidney disease and for the use of zvegf4 antagonists, including antibodies, in the treatment of kidney disease.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

3.30.06

Date

Debra G. Gilbertson

Herceptin
Trastuzumab

WARNINGS:
CARDIOMYOPATHY
HERCEPTIN administration can result in the development of ventricular dysfunction and congestive heart failure. Left ventricular function should be evaluated in all patients prior to and during treatment with HERCEPTIN. Discontinuation of HERCEPTIN treatment should be strongly considered in patients who develop a clinically significant decrease in left ventricular function. The incidence and severity of cardiac dysfunction was particularly high in patients who received HERCEPTIN in combination with anthracyclines and cyclophosphamide. (See WARNINGS.)

HYPERSENSITIVITY REACTIONS INCLUDING ANAPHYLAXIS
INFUSION REACTIONS
PULMONARY EVENTS
HERCEPTIN administration can result in severe hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events. Rarely, these have been fatal. In most cases, symptoms occurred during or within 24 hours of administration of HERCEPTIN. HERCEPTIN infusion should be interrupted for patients experiencing dyspnea or clinically significant hypotension. Patients should be monitored until signs and symptoms completely resolve. Discontinuation of HERCEPTIN treatment should be strongly considered for patients who develop anaphylaxis, angioedema, or acute respiratory distress syndrome. (See WARNINGS.)

DESCRIPTION
HERCEPTIN (Trastuzumab) is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay (Kd=5 nM) to the extracellular domain of the human epidermal growth factor receptor 2 protein, HER2.[1,2] The antibody is an IgG$_1$ kappa that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2.

The humanized antibody against HER2 is produced by a mammalian cell (Chinese Hamster Ovary [CHO]) suspension culture in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product.

HERCEPTIN is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. The nominal content of each HERCEPTIN vial is 440 mg Trastuzumab, 400 mg α, α-trehalose dihydrate, 9.9 mg L-histidine HCl, 6.4 mg L-histidine, and 1.8 mg polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI), USP, containing 1.1% benzyl alcohol as a preservative, yields a multi-dose solution containing 21 mg/mL Trastuzumab, at a pH of approximately 6.

CLINICAL PHARMACOLOGY
General
The HER2 (or c-erbB2) proto-oncogene encodes a transmembrane receptor protein of 185 kDa, which is structurally related to the epidermal growth factor receptor.[3] HER2 protein overexpression is observed in 25%–30% of primary breast cancers. HER2 protein overexpression can be determined using immunohistochemistry (IHC) and gene amplification can be determined using fluorescence *in situ* hybridization (FISH) of fixed tumor blocks.[4] In referenced studies where HERCEPTIN use was not studied,[3-5] approximately 95%–98% of biopsy specimens that were found to have protein overexpression also had gene amplification and 100% of those with gene amplification also had protein overexpression.[3-5] The precision of the determination of protein overexpression or gene amplification, however, may vary depending on the sensitivity and specificity of the particular assay and assay procedures used (see PRECAUTIONS). When compared to the referenced studies noted above, the correlation between detectable protein overexpression using IHC and detectable gene amplification using FISH was not as high in the studies of HERCEPTIN clinical trial specimens (see CLINICAL STUDIES: HER2 Detection and HER2 Assay Concordance Studies, and PRECAUTIONS: HER2 Testing).

Trastuzumab has been shown, in both *in vitro* assays and in animals, to inhibit the proliferation of human tumor cells that overexpress HER2.[1,6]

Trastuzumab is a mediator of antibody-dependent cellular cytotoxicity (ADCC).[1,6] *In vitro*, HERCEPTIN-mediated ADCC has been shown to be preferentially exerted on HER2 overexpressing cancer cells compared with cancer cells that do not overexpress HER2.

Pharmacokinetics
The pharmacokinetics of Trastuzumab were studied in breast cancer patients with metastatic disease. Short duration intravenous infusions of 10 to 500 mg once weekly demonstrated dose-dependent pharmacokinetics. Mean half-life increased and clearance decreased with increasing dose level. The half-life averaged 1.7 and 12 days at the 10 and 500 mg dose levels, respectively. Trastuzumab's volume of distribution was approximately that of serum volume (44 mL/kg). At the highest weekly dose studied (500 mg), mean peak serum concentrations were 377 µg/mL.

In studies using a loading dose of 4 mg/kg followed by a weekly maintenance dose of 2 mg/kg, a mean half-life of 5.8 days (range=1 to 32 days) was observed. Between Weeks 16 and 32, Trastuzumab serum concentrations reached a steady state with mean trough and peak concentrations of approximately 79 µg/mL and 123 µg/mL, respectively.

Detectable concentrations of the circulating extracellular domain of the HER2 receptor (shed antigen) are found in the sera of some patients with HER2 overexpressing tumors. Determination of shed antigen in baseline serum samples revealed that 54% (286/447) of patients had detectable shed antigen, which ranged as high as 1880 ng/mL (median=11 ng/mL). Patients with higher baseline shed antigen levels were more likely to have lower serum trough concentrations. However, with weekly dosing, most patients with elevated shed antigen levels achieved target serum concentrations of Trastuzumab by Week 6.

Data suggest that the disposition of Trastuzumab is not altered based on age or serum creatinine (up to 2.0 mg/dL). No formal interaction studies have been performed.

Mean serum trough concentrations of Trastuzumab, when administered in combination with paclitaxel, were consistently elevated approximately 1.5-fold as compared with serum concentrations of Trastuzumab used in combination with anthracycline plus cyclophosphamide. In primate studies, administration of Trastuzumab with paclitaxel resulted in a reduction in Trastuzumab clearance. Serum levels of Trastuzumab in combination with cisplatin, doxorubicin or epirubicin plus cyclophosphamide did not suggest any interactions; no formal drug interaction studies were performed.

CLINICAL STUDIES
The safety and efficacy of HERCEPTIN were studied in a randomized, controlled clinical trial in combination with chemotherapy (469 patients) and an open-label single agent clinical trial (222 patients). Both trials studied patients with metastatic breast cancer whose tumors overexpress the [illegible] protein. Patients were eligible if they had 2+ or 3+ levels of overexpression (based on a 0 to 3+ scale) by immunohistochemical assessment of tumor tissue performed by a central testing lab.

A multicenter, randomized, controlled clinical trial was conducted in 469 patients with metastatic breast cancer who had not been previously treated with chemotherapy for metastatic disease.[7] Patients were randomized to receive chemotherapy alone or in combination with HERCEPTIN given intravenously as a 4 mg/kg loading dose followed by weekly doses of HERCEPTIN at 2 mg/kg. For those who had received prior anthracycline therapy in the adjuvant setting, chemotherapy consisted of paclitaxel (175 mg/m^2 over 3 hours every 21 days for at least six cycles); for all other patients, chemotherapy consisted of anthracycline plus cyclophosphamide (AC: doxorubicin 60 mg/m^2 or epirubicin 75 mg/m^2 plus 600 mg/m^2 cyclophosphamide every 21 days for six cycles). Compared with patients in the AC subgroups (n=281), patients in the paclitaxel subgroup (n=185) were more likely to have had the following: poor prognostic factors (premenopausal status, estrogen or progesterone receptor negative tumors, positive lymph nodes), prior therapy (adjuvant chemotherapy, myeloablative chemotherapy, radiotherapy), and a shorter disease-free interval. Sixty-five percent of patients randomized to receive chemotherapy alone in this study received HERCEPTIN at the time of disease progression as part of a separate extension study.

Compared with patients randomized to chemotherapy alone, the patients randomized to HERCEPTIN and chemotherapy experienced a significantly longer median time to disease progression, a higher overall response rate (ORR), a longer median duration of response, and a longer median survival (see Table 1). These treatment effects were observed both in patients who received HERCEPTIN plus paclitaxel and in those who received HERCEPTIN plus AC; however the magnitude of the effects was greater in the paclitaxel subgroup (see CLINICAL STUDIES: HER2 Detection).

Table 1
Phase III Clinical Efficacy in First-Line Treatment

	Combined Results		Paclitaxel Subgroup		AC Subgroup	
	HERCEPTIN + All Chemotherapy (n = 235)	All Chemotherapy (n = 234)	HERCEPTIN + Paclitaxel (n = 92)	Paclitaxel (n = 96)	HERCEPTIN + AC[a] (n = 143)	AC (n = 138)
Primary Endpoint						
Time to Progression[b,c]						
Median (months)	7.2	4.5	6.7	2.5	7.6	5.7
95% confidence interval	6.9, 8.2	4.3, 4.9	5.2, 9.8	2.0, 4.3	7.2, 9.1	4.6, 7.1
p-value (log rank)	<0.0001		<0.0001		0.002	
Secondary Endpoints						
Overall Response Rate[b]						
Rate (percent)	45	29	36	15	50	39
95% confidence interval	39, 51	23, 35	26, 48	8, 22	42, 58	30, 48
p-value (χ^2-test)	<0.001		<0.001		0.10	
Duration of Response[b,c]						
Median (months)	8.3	5.8	8.3	4.3	8.4	6.4
25%, 75% quartile	5.5, 14.8	3.0, 8.5	5.1, 11.0	3.7, 7.4	5.8, 14.8	4.5, 8.5
Survival Time[b]						
Median Survival (months)	25.1	20.3	22.1	18.4	25.8	21.4
95% confidence interval	22.2, 29.5	16.8, 24.2	18.9, 28.6	12.7, 24.4	23.3, 32.9	16.3, 26.6
p-value (log rank)	0.05		0.17		0.16	

[a] AC = Anthracycline (doxorubicin or epirubicin) and cyclophosphamide.
[b] Assessed by an Independent Response Evaluation Committee.
[c] Kaplan-Meier Estimate.

HERCEPTIN was studied as a single agent in a multicenter, open-label, single-arm clinical trial in patients with HER2 overexpressing metastatic breast cancer who had relapsed following one o two prior chemotherapy regimens for metastatic disease. Of 222 patients enrolled, 65% had received prior adjuvant chemotherapy, 68% had received two prior chemotherapy regimens fo metastatic disease, and 25% had received prior myeloablative treatment with hematopoietic rescue. Patients were treated with a loading dose of 4 mg/kg IV followed by weekly doses o HERCEPTIN at 2 mg/kg IV. The ORR (complete response + partial response), as determined by an Independent Response Evaluation Committee, was 14%, with a 2% complete response rate and a 12% partial response rate. Complete responses were observed only in patients with disease limited to skin and lymph nodes (see CLINICAL STUDIES: HER2 Detection).

HER2 Detection
(See PRECAUTIONS: HER2 Testing)
Detection of HER2 protein overexpression is necessary for selection of patients appropriate for HERCEPTIN therapy (see INDICATIONS AND USAGE). Overexpression of HER2 by tumors was an entry criterion of the two clinical studies described above. In those studies, a research-use-only IHC assay (referred to as the Clinical Trial Assay [CTA]) was used.

The commercial assays described below, HercepTest® (IHC assay) and PathVysion® (FISH assay) are appropriate assays to aid in the selection of patients for HERCEPTIN therapy (see HER2 Protein Overexpression Detection Methods and HER2 Gene Amplification Detection Methods). The comparability of either assay with regard to the ability to predict clinical benefit from HERCEPTIN therapy has not been prospectively studied. In addition, the utility of either assay in patients whose tumors would score as 0 or 1+ by the CTA has not been established because patients with tumors that scored as 0 or 1+ were excluded from the clinical studies described.

HER2 Protein Overexpression Detection Methods
HER2 protein overexpression can be established by measuring expressed HER2 protein using IHC methodology. In the clinical trial studies described above, specimens were tested with the CTA and scored as 0, 1+, 2+, or 3+ with 3+ indicating the strongest positivity. Only patients with 2+ or 3+ positive tumors were eligible (about 33% of those screened). Data from the randomized trial suggest that the beneficial treatment effects were largely limited to patients with the highest level of HER2 protein overexpression (3+) (see Table 2). In an exploratory analysis, the relative risk (π) for time to progression was lower in the patients whose tumors tested as CTA 3+ (π = 0.42 with 95% CI: 0.33, 0.54) than in those tested as CTA 2+ (π = 0.76 with 95% CI: 0.50, 1.15). The relative risk represents the risk of progression in the HERCEPTIN plus chemotherapy arm versus the chemotherapy arm. Therefore, a lower ratio represents longer time to progression in the HERCEPTIN arm. In the single arm study of HERCEPTIN as a single agent, the overall response rate in patients whose tumors tested as CTA 3+ was 18% while in those that tested as CTA 2+, it was 6%.

HercepTest®, another IHC assay, was assessed for concordance with the CTA (see HER2 Assay Concordance Studies), but has not been used to assess tumor specimens from the HERCEPTIN clinical studies described above.

HER2 Gene Amplification Detection Methods
As a surrogate for protein overexpression, measurement of the number of HER2 gene copies using FISH to detect gene amplification may be employed. An exploratory, retrospective assessment of known CTA 2+ or 3+ tumor specimens was performed to detect HER2 gene amplification using PathVysion®, a FISH assay. Data from this retrospective analysis involving 660 of 691 (96%) patients enrolled in the clinical studies (all scoring 2+ or 3+ by the CTA) suggested that the beneficial treatment effects were greater in patients whose tumors tested as FISH (+) than in those that were FISH (-); however, time to progression was prolonged for patients on the HERCEPTIN arm, regardless of the FISH result (see Table 2). In the single arm study of HERCEPTIN as a single agent, the overall response rate in patients whose tumors tested as FISH (+) was 20%, while in those tested as FISH (-), there were no responses.

These data are not sufficient to conclude whether FISH testing can distinguish a subpopulation of CTA 2+ patients who would be unlikely to benefit from HERCEPTIN therapy. In addition, there are no data correlating clinical outcome with FISH test results for patients with tumors that scored as 0 or 1+ by CTA; therefore, conclusions regarding the usefulness of FISH in the general population cannot be made.

Table 2
Treatment Effect versus Level of HER2 Expression
Phase III Randomized Trial (N = 469):
HERCEPTIN Plus Chemotherapy versus Chemotherapy

HER2 Assay Result	Number of Patients (N)	Relative Risk** for Time to Disease Progression (95% CI)	Relative Risk** for Mortality (95% CI)
CTA 2+ or 3+	469	0.49 (0.40, 0.61)	0.80 (0.64, 1.00)
FISH (+)*	325	0.44 (0.34, 0.57)	0.70 (0.53, 0.91)
FISH (-)*	126	0.62 (0.42, 0.94)	1.05 (0.70, 1.63)
CTA 2+	120	0.78 (0.50, 1.15)	1.28 (0.82, 1.94)
FISH (+)	32	0.54 (0.21, 1.35)	1.31 (0.53, 3.27)
FISH (-)	83	0.77 (0.48, 1.25)	1.11 (0.68, 1.82)
CTA 3+	349	0.42 (0.33, 0.54)	0.70 (0.51, 0.90)
FISH (+)	293	0.42 (0.32, 0.56)	0.67 (0.51, 0.89)
FISH (-)	43	0.43 (0.20, 0.84)	0.88 (0.39, 1.98)

*FISH testing results were available for 451 of the 469 patients enrolled on study.
**The relative risk represents the risk of progression or death in the HERCEPTIN plus chemotherapy arm versus the chemotherapy arm.

HER2 Assay Concordance Studies
(See PRECAUTIONS: HER2 Testing)

Immunohistochemistry: The DAKO HercepTest®, an IHC test for detecting HER2 protein overexpression, has not been directly studied for its ability to predict HERCEPTIN treatment effect, but has been compared to the CTA on over 500 breast cancer histology specimens obtained from the National Cancer Institute Cooperative Breast Cancer Tissue Resource. Based upon these results, of specimens testing 3+ (strongly positive) on the HercepTest®, 82% were 3+ (i.e., the reading most associated with clinical benefit), 12% were 2+, and 6% were 0 or 1+ on the CTA. The 6% of HercepTest® 3+ specimens that were CTA 0 or 1+ would be expected to represent 2% of the 0 and 1+ population. Of specimens testing 2+ (weakly positive) on the HercepTest®, 14% were 3+, 20% were 2+, and 66% were 0 or 1+ on the CTA. Of specimens testing 0 or 1+ on the HercepTest®, 2% were 3+, 6% were 2+, and 92% were 0 or 1+ on the CTA.

Fluorescence In Situ Hybridization: The Vysis PathVysion® HER2 DNA Probe, a FISH test for detecting HER2 gene amplification, was compared with the CTA on over 500 breast cancer histology specimens originally submitted for potential enrollment in the HERCEPTIN trials. A HER2:CEP17 ratio of ≥2 was defined as FISH positive (+). Based on these results, of specimens testing FISH (+) by PathVysion®, 81% were 3+, 10% were 2+, and 9% were 0 or 1+ on the CTA. The 9% of FISH (+) specimens that were CTA 0 or 1+ would be expected to represent 3% of the total CTA 0 or 1+ population. Of specimens testing FISH (-) by PathVysion®, 3% were 3+, 10% were 2+, and 87% were 0 or 1+ on the CTA.

INDICATIONS AND USAGE
HERCEPTIN (Trastuzumab) as a single agent is indicated for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have received one or more chemotherapy regimens for their metastatic disease. HERCEPTIN in combination with paclitaxel is indicated for treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have not received chemotherapy for their metastatic disease. HERCEPTIN should be used in patients whose tumors have been evaluated with an assay validated to predict HER2 protein overexpression (see PRECAUTIONS: HER2 Testing and CLINICAL STUDIES: HER2 Detection).

CONTRAINDICATIONS
None known.

WARNINGS
Cardiotoxicity
Signs and symptoms of cardiac dysfunction, such as dyspnea, increased cough, paroxysmal nocturnal dyspnea, peripheral edema, S_3 gallop, or reduced ejection fraction, have been observed in patients treated with HERCEPTIN. Congestive heart failure associated with HERCEPTIN therapy may be severe and has been associated with disabling cardiac failure, death, and mural thrombosis leading to stroke (see BOXED WARNINGS: CARDIOMYOPATHY). The clinical status of patients in the trials who developed congestive heart failure was classified for severity using the New York Heart Association classification system (I-IV, where IV is the most severe level of cardiac failure) (see Table 3).

Table 3
Incidence and Severity of Cardiac Dysfunction

	HERCEPTIN* Alone n=213	HERCEPTIN + Paclitaxel* n=91	Paclitaxel* n=85	HERCEPTIN + Anthracycline + Cyclophosphamide† n=143	Anthracycline + Cyclophosphamide† n=135
Any Cardiac Dysfunction	7%	11%	1%	28%	7%
Class III-IV	5%	4%	1%	19%	3%

*Open-label, single-agent Phase II study (94% received prior anthracyclines).
†Randomized Phase III study comparing chemotherapy plus HERCEPTIN to chemotherapy alone, where chemotherapy is either anthracycline/cyclophosphamide or paclitaxel.

Candidates for treatment with HERCEPTIN should undergo thorough baseline cardiac assessment including history and physical exam and one or more of the following: EKG, echocardiogram, and MUGA scan. There are no data regarding the most appropriate method of evaluation for the identification of patients at risk for developing cardiotoxicity. Monitoring may not identify all patients who will develop cardiac dysfunction.

Cardiac function should be evaluated in treating patients with pre-existing cardiac dysfunction.

Patients receiving HERCEPTIN should undergo frequent monitoring for deteriorating cardiac function.

The probability of cardiac dysfunction was highest in patients who received HERCEPTIN concurrently with anthracyclines. The data suggest that advanced age may increase the probability of cardiac dysfunction.

Pre-existing cardiac disease or prior cardiotoxic therapy (e.g., anthracycline or radiation therapy to the chest) may decrease the ability to tolerate HERCEPTIN therapy; however, the data are not adequate to evaluate the correlation between HERCEPTIN-induced cardiotoxicity and these factors.

Discontinuation of HERCEPTIN therapy should be strongly considered in patients who develop clinically significant congestive heart failure. In the clinical trials, most patients with cardiac dysfunction responded to appropriate medical therapy often including discontinuation of HERCEPTIN. The safety of continuation or resumption of HERCEPTIN in patients who have previously experienced cardiac toxicity has not been studied. There are insufficient data regarding discontinuation of HERCEPTIN therapy in patients with asymptomatic decreases in ejection fraction; such patients should be closely monitored for evidence of clinical deterioration.

Hypersensitivity Reactions Including Anaphylaxis
Severe hypersensitivity reactions have been infrequently reported in patients treated with HERCEPTIN (see BOXED WARNINGS: HYPERSENSITIVITY REACTIONS INCLUDING ANAPHYLAXIS). Signs and symptoms include anaphylaxis, urticaria, bronchospasm, angioedema, and/or hypotension. In some cases, the reactions have been fatal. The onset of symptoms generally occurred during an infusion but there have also been reports of symptom onset after the completion of an infusion. Reactions were most commonly reported in association with the initial infusion.

HERCEPTIN infusion should be interrupted in all patients with severe hypersensitivity reactions. In the event of a hypersensitivity reaction, appropriate medical therapy should be administered, which may include epinephrine, corticosteroids, diphenhydramine, bronchodilators and oxygen. Patients should be evaluated and carefully monitored until complete resolution of signs and symptoms.

There are no data regarding the most appropriate method of identification of patients who may safely be retreated with HERCEPTIN after experiencing a severe hypersensitivity reaction. HERCEPTIN has been readministered to some patients who fully recovered from a previous severe reaction. Prior to readministration of HERCEPTIN, the majority of these patients were prophylactically treated with pre-medications including antihistamines and/or corticosteroids. While some of these patients tolerated retreatment, others had severe reactions again despite the use of prophylactic pre-medications.

Infusion Reactions
In the postmarketing setting, rare occurrences of severe infusion reactions leading to a fatal outcome have been associated with the use of HERCEPTIN (see BOXED WARNINGS: INFUSION REACTIONS).

In clinical trials, infusion reactions consisted of a symptom complex characterized by fever and chills, and on occasion included nausea, vomiting, pain (in some cases at tumor sites), headache, dizziness, dyspnea, hypotension, rash, and asthenia. These reactions were usually mild to moderate in severity (see ADVERSE REACTIONS).

However, in postmarketing reports, more severe adverse reactions to HERCEPTIN infusion were observed and included bronchospasm, hypoxia, and severe hypotension. These severe reactions were usually associated with the initial infusion of HERCEPTIN and generally occurred during or immediately following the infusion. However, the onset and clinical course were variable. For some patients, symptoms progressively worsened and led to further pulmonary complications (see WARNINGS: Pulmonary Events). In other patients with acute onset of signs and symptoms, initial improvement was followed by clinical deterioration. Delayed post-infusion events with rapid clinical deterioration have also been reported. Rarely, severe infusion reactions culminated in death within hours or up to one week following an infusion.

Some severe reactions have been treated successfully with interruption of the HERCEPTIN infusion and supportive therapy including oxygen, intravenous fluids, beta-agonists, and corticosteroids.

There are no data regarding the most appropriate method of identification of patients who may safely be retreated with HERCEPTIN after experiencing a severe infusion reaction. HERCEPTIN has been readministered to some patients who fully recovered from the previous severe reaction. Prior to readministration of HERCEPTIN, the majority of these patients were prophylactically treated with pre-medications including antihistamines and/or corticosteroids. While some of these patients tolerated retreatment, others had severe reactions again despite the use of prophylactic pre-medications.

Exacerbation of Chemotherapy-Induced Neutropenia
In randomized, controlled clinical trials designed to assess the impact of the addition of HERCEPTIN on chemotherapy, the per-patient incidences of moderate to severe neutropenia and of febrile neutropenia were higher in patients receiving HERCEPTIN in combination with myelosuppressive chemotherapy as compared to those who received chemotherapy alone. In the postmarketing setting, deaths due to sepsis in patients with severe neutropenia have been reported in patients receiving HERCEPTIN and myelosuppressive chemotherapy, although in controlled clinical trials (pre- and post-marketing), the incidence of septic deaths was not significantly increased. The pathophysiologic basis for exacerbation of neutropenia has not been determined, the effect of HERCEPTIN on the pharmacokinetics of chemotherapeutic agents has not been fully evaluated (see ADVERSE REACTIONS: Anemia and Leukopenia; ADVERSE REACTIONS: Infection).

Pulmonary Events
Severe pulmonary events leading to death have been reported rarely with the use of HERCEPTIN in the postmarketing setting. Signs, symptoms and clinical findings include dyspnea, pulmonary infiltrates, pleural effusions, non-cardiogenic pulmonary edema, pulmonary insufficiency and hypoxia, and acute respiratory distress syndrome. These events may or may not occur as sequela of infusion reactions (see WARNINGS: Infusion Reactions). Patients with symptomatic intrinsic lung disease or with extensive tumor involvement of the lungs, resulting in dyspnea at rest, may be at greater risk of severe reactions.

Other severe events reported rarely in the postmarketing setting include pneumonitis and pulmonary fibrosis.

PRECAUTIONS
General
HERCEPTIN therapy should be used with caution in patients with known hypersensitivity to Trastuzumab, Chinese Hamster Ovary cell proteins, or any component of this product.

HER2 Testing
Assessment for HER2 overexpression should be performed by laboratories with demonstrated proficiency in the specific technology being utilized. Improper assay performance, including use of suboptimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results. Refer to the HercepTest® and PathVysion® package inserts for full instructions on assay performance (see CLINICAL STUDIES: HER2 Detection).

Drug Interactions
There have been no formal drug interaction studies performed with HERCEPTIN in humans. Administration of paclitaxel in combination with HERCEPTIN resulted in a two-fold decrease in HERCEPTIN clearance in a non-human primate study and in a 1.5-fold increase in HERCEPTIN serum levels in clinical studies (see CLINICAL PHARMACOLOGY: Pharmacokinetics).

Benzyl Alcohol
For patients with a known hypersensitivity to benzyl alcohol (the preservative in Bacteriostatic Water for Injection) reconstitute HERCEPTIN with Sterile Water for Injection (SWFI), USP. DISCARD THE SWFI-RECONSTITUTED HERCEPTIN VIAL FOLLOWING A SINGLE USE.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis
HERCEPTIN has not been tested for its carcinogenic potential.

Mutagenesis
No evidence of mutagenic activity was observed in Ames tests using six different test strains of bacteria, with and without metabolic activation, at concentrations of up to 5000 µg/mL Trastuzumab. Human peripheral blood lymphocytes treated *in vitro* at concentrations of up to 5000 µg/plate Trastuzumab, with and without metabolic activation, revealed no evidence of mutagenic potential. In an *in vivo* mutagenic assay (the micronucleus assay), no evidence of chromosomal damage to mouse bone marrow cells was observed following bolus intravenous doses of up to 118 mg/kg Trastuzumab.

Impairment of Fertility
A fertility study has been conducted in female cynomolgus monkeys at doses up to 25 times the weekly human maintenance dose of 2 mg/kg HERCEPTIN and has revealed no evidence of impaired fertility.

Pregnancy Category B
There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, HERCEPTIN should be used during pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus.

In the postmarketing setting, oligohydramnios has been reported in women who received HERCEPTIN during pregnancy, either in combination with chemotherapy or as a single agent. Given the limited number of reported cases, the high background rate of occurrence of oligohydramnios, the lack of clear temporal relationships between drug use and clinical findings, and the lack of supportive findings in animal studies, an association between HERCEPTIN and oligohydramnios has not been established.

Reproduction studies have been conducted in cynomolgus monkeys at doses up to 25 times the weekly human maintenance dose of 2 mg/kg HERCEPTIN and have revealed no evidence of impaired fertility or harm to the fetus. However, HER2 protein expression is high in many embryonic tissues including cardiac and neural tissues; in mutant mice lacking HER2, embryos died in early gestation.[8] Placental transfer of HERCEPTIN during the early (Days 20–50 of gestation) and late (Days 120–150 of gestation) fetal development period was observed in monkeys.

Nursing Mothers
A study conducted in lactating cynomolgus monkeys at doses 25 times the weekly human maintenance dose of 2 mg/kg HERCEPTIN demonstrated that Trastuzumab is secreted in the milk. The presence of Trastuzumab in the serum of infant monkeys was not associated with any adverse effects on their growth or development from birth to 3 months of age. It is not known whether HERCEPTIN is secreted in human milk. Because human IgG is secreted in human milk, and the potential for absorption and harm to the infant is unknown, women should be advised to discontinue nursing during HERCEPTIN therapy and for 6 months after the last dose of HERCEPTIN.

Pediatric Use
The safety and effectiveness of HERCEPTIN in pediatric patients have not been established.

Geriatric Use
HERCEPTIN has been administered to 133 patients who were 65 years of age or over. The risk of cardiac dysfunction may be increased in geriatric patients. The reported clinical experience is not adequate to determine whether older patients respond differently from younger patients.

ADVERSE REACTIONS
The most serious adverse reactions caused by HERCEPTIN include cardiomyopathy, hypersensitivity reactions including anaphylaxis, infusion reactions, pulmonary events, and exacerbation of chemotherapy-induced neutropenia. Please refer to the BOXED WARNINGS and/or WARNINGS sections for detailed descriptions of these reactions. The most common adverse reactions associated with HERCEPTIN use are fever, diarrhea, infections, chills, increased cough, headache, rash, and insomnia.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to drug use and for approximating rates.

Additional adverse reactions have been identified during post-marketing use of HERCEPTIN. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to HERCEPTIN exposure. Decisions to include these reactions in labeling are typically based on one or more of the following factors: (1) seriousness of the reaction, (2) frequency of reporting, or (3) strength of causal connection to HERCEPTIN.

Where specific percentages are noted, these data are based on clinical studies of HERCEPTIN alone or in combination with chemotherapy in clinical trials. Data in Table 4 are based on the experience with the recommended dosing regimen for HERCEPTIN in a randomized controlled clinical trial of 234 patients who received HERCEPTIN in combination with chemotherapy and four open-label studies of HERCEPTIN as a single agent in 352 patients at doses of 10–500 mg administered weekly. Data regarding serious adverse events are based on experience in 958 patients enrolled in all clinical trials of HERCEPTIN conducted prior to marketing approval.

Cardiac Failure/Dysfunction
For a description of cardiac toxicities, see BOXED WARNINGS: CARDIOMYOPATHY and WARNINGS: Cardiotoxicity.

Anemia and Leukopenia
In a randomized, controlled trial (see CLINICAL STUDIES), the per-patient incidences of anemia (30% vs. 21%) and leukopenia (53% vs. 37%) were higher in patients receiving HERCEPTIN in combination with chemotherapy as compared to those receiving chemotherapy alone. The majority of these cytopenic events were mild to moderate in intensity, reversible, and none resulted in discontinuation of therapy with HERCEPTIN.

In a randomized, controlled trial conducted in the post-marketing setting, there were also increased incidences of NCI-CTC Grade 3/4 neutropenia (32% [29/92] vs. 22% [21/94]) and of febrile neutropenia (23% [21/91] vs. 17% [16/94]) in patients randomized to HERCEPTIN in combination with myelosuppressive chemotherapy as compared to chemotherapy alone (see ADVERSE REACTIONS: Infection).

Hematologic toxicity is infrequent following the administration of HERCEPTIN as a single agent, with an incidence of Grade III toxicities for WBC, platelets, hemoglobin all <1%. No Grade IV toxicities were observed.

Diarrhea
Of patients treated with HERCEPTIN as a single agent, 25% experienced diarrhea. An increased incidence of diarrhea, primarily mild to moderate in severity, was observed in patients receiving HERCEPTIN in combination with chemotherapy.

Infection
In a randomized, controlled trial (see CLINICAL STUDIES), the incidence of infections, primarily mild upper respiratory infections of minor clinical significance or urinary infections, was higher [40% vs. 30%] in patients receiving HERCEPTIN in combination with chemotherapy as compared to those receiving chemotherapy alone.

In a randomized, controlled trial conducted in the post-marketing setting, the reported incidence of febrile neutropenia was higher (23% [21/92] vs. 17% [16/94]) in patients receiving HERCEPTIN in combination with myelosuppressive chemotherapy as compared to chemotherapy alone.

In the postmarketing setting, there have also been reports of febrile neutropenia and infection with neutropenia culminating in death associated with the use of HERCEPTIN and myelosuppressive chemotherapy (see WARNINGS: Exacerbation of Chemotherapy-Induced Neutropenia).

Infusion Reactions
During the first infusion with HERCEPTIN, a symptom complex most commonly consisting of chills and/or fever was observed in about 40% of patients in clinical trials. The symptoms were usually mild to moderate in severity and were treated with acetaminophen, diphenhydramine, and meperidine (with or without reduction in the rate of HERCEPTIN infusion). HERCEPTIN discontinuation was infrequent. Other signs and/or symptoms may include nausea, vomiting, pain (in some cases at tumor sites), rigors, headache, dizziness, dyspnea, hypotension, elevated blood pressure, rash, and asthenia. The symptoms occurred infrequently with subsequent HERCEPTIN infusions (see BOXED WARNINGS: INFUSION REACTIONS and WARNINGS: Infusion Reactions).

Hypersensitivity Reactions Including Anaphylaxis
Pulmonary Events
In the postmarketing setting, severe hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary adverse events have been reported (see BOXED WARNINGS: HYPERSENSITIVITY REACTIONS INCLUDING ANAPHYLAXIS and WARNINGS: Hypersensitivity Reactions including Anaphylaxis). These events include anaphylaxis, angioedema, bronchospasm, hypotension, hypoxia, dyspnea, pulmonary infiltrates, pleural effusions, non-cardiogenic pulmonary edema, and acute respiratory distress syndrome. For a detailed description, see WARNINGS.

Glomerulopathy
In the postmarketing setting, rare cases of nephrotic syndrome with pathologic evidence of glomerulopathy have been reported. The time to onset ranged from 4 months to approximately 1 months from initiation of HERCEPTIN therapy. Pathologic findings included membranous glomerulonephritis, focal glomerulosclerosis, and fibrillary glomerulonephritis. Complications included volume overload and congestive heart failure.

Table 4
Adverse Events Occurring in ≥5% of Patients or at Increased Incidence in the HERCEPTIN Arm of the Randomized Study (Percent of Patients)

	Single Agent n=352	HERCEPTIN + Paclitaxel n=91	Paclitaxel Alone n=95	HERCEPTIN + AC n=143	AC Alone n=135
Body as a Whole					
Pain	47	61	62	57	42
Asthenia	42	52	57	54	55
Fever	36	49	23	56	34
Chills	32	41	4	35	11
Headache	26	36	28	44	31
Abdominal pain	22	34	22	23	18
Back pain	22	34	30	27	15
Infection	20	47	27	47	31
Flu syndrome	10	12	5	12	8
Accidental injury	6	13	3	9	4
Allergic reaction	9	8	2	4	2
Cardiovascular					
Tachycardia	5	12	4	10	5
Congestive heart failure	7	11	1	28	7
Digestive					
Nausea	33	51	9	76	77
Diarrhea	25	45	20	45	26
Vomiting	23	37	28	53	49
Nausea and vomiting	8	14	11	18	9
Anorexia	14	24	10	31	26
Heme & Lymphatic					
Anemia	4	14	9	36	26
Leukopenia	3	24	17	52	34
Metabolic					
Peripheral edema	10	22	20	20	17
Edema	8	10	8	11	5
Musculoskeletal					
Bone pain	7	24	18	7	7
Arthralgia	6	37	21	8	9
Nervous					
Insomnia	14	25	13	29	15
Dizziness	13	22	24	24	18
Paresthesia	9	18	30	17	11
Depression	6	12	13	20	12
Peripheral neuritis	2	23	16	2	2
Neuropathy	1	13	5	4	4
Respiratory					
Cough increased	26	41	22	43	29
Dyspnea	22	27	26	42	25
Rhinitis	14	22	5	22	16
Pharyngitis	12	22	14	30	18
Sinusitis	9	21	7	13	6
Skin					
Rash	18	38	18	27	17
Herpes simplex	2	12	3	7	9
Acne	2	11	3	3	<1
Urogenital					
Urinary tract infection	5	18	14	13	7

Other Serious Adverse Events
The following other serious adverse events occurred in at least one of the 956 patients treated with HERCEPTIN in clinical studies:

Body as a Whole: cellulitis, anaphylactoid reaction, ascites, hydrocephalus, radiation injury, deafness, amblyopia

Cardiovascular: vascular thrombosis, pericardial effusion, heart arrest, hypotension, syncope, hemorrhage, shock, arrhythmia

Digestive: hepatic failure, gastroenteritis, hematemesis, ileus, intestinal obstruction, colitis, esophageal ulcer, stomatitis, pancreatitis, hepatitis

Endocrine: hypothyroidism

Hematological: pancytopenia, acute leukemia, coagulation disorder, lymphangitis

Metabolic: hypercalcemia, hypomagnesemia, hyponatremia, hypoglycemia growth retardation, weight loss

Musculoskeletal: pathological fractures, bone necrosis, myopathy

Nervous: convulsion, ataxia, confusion, manic reaction

Respiratory: apnea, pneumothorax, asthma, hypoxia, laryngitis

Skin: herpes zoster, skin ulceration

Urogenital: hydronephrosis, kidney failure, cervical cancer, hematuria, hemorrhagic cystitis, pyelonephritis

Immunogenicity
Of 903 patients who have been evaluated, human anti-human antibody (HAHA) to Trastuzumab was detected in one patient, who had no allergic manifestations.

The data reflect the percentage of patients whose test results were considered positive for antibodies to HERCEPTIN in the HAHA assay for Trastuzumab, and are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in an assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to HERCEPTIN with the incidence of antibodies to other products may be misleading.

OVERDOSAGE
There is no experience with overdosage in human clinical trials. Single doses higher than 500 mg have not been tested.

DOSAGE AND ADMINISTRATION
Usual Dose
The recommended initial loading dose is 4 mg/kg Trastuzumab administered as a 90-minute infusion. The recommended weekly maintenance dose is 2 mg/kg Trastuzumab and can be administered as a 30-minute infusion if the initial loading dose was well tolerated. HERCEPTIN may be administered in an outpatient setting. HERCEPTIN is to be diluted in saline for IV infusion. **DO NOT ADMINISTER AS AN IV PUSH OR BOLUS.** (See DOSAGE AND ADMINISTRATION: Administration.)

Preparation for Administration
The diluent provided has been formulated to maintain the stability and sterility of HERCEPTIN for up to 28 days. Other diluents have not been shown to contain effective preservatives for HERCEPTIN. Each vial of HERCEPTIN should be reconstituted with 20 mL of BWFI, USP, 1.1% benzyl alcohol preserved, as supplied, to yield a multi-dose solution containing 21 mg/mL Trastuzumab. Immediately upon reconstitution with BWFI, the vial of HERCEPTIN must be labeled in the area marked "Do not use after:" with the future date that is 28 days from the date of reconstitution.

If the patient has known hypersensitivity to benzyl alcohol, HERCEPTIN must be reconstituted with Sterile Water for Injection (see PRECAUTIONS). HERCEPTIN WHICH HAS BEEN RECONSTITUTED WITH SWFI MUST BE USED IMMEDIATELY AND ANY UNUSED PORTION DISCARDED. USE OF OTHER RECONSTITUTION DILUENTS SHOULD BE AVOIDED.

Shaking the reconstituted HERCEPTIN or causing excessive foaming during the addition of diluent may result in problems with dissolution and the amount of HERCEPTIN that can be withdrawn from the vial.

Use appropriate aseptic technique when performing the following reconstitution steps:

a. Using a sterile syringe, slowly inject the 20 mL of diluent into the vial containing the lyophilized cake of Trastuzumab. The stream of diluent should be directed into the lyophilized cake.

b. Swirl the vial gently to aid reconstitution. Trastuzumab may be sensitive to shear-induced stress, e.g., agitation or rapid expulsion from a syringe. **DO NOT SHAKE.**

c. Slight foaming of the product upon reconstitution is not unusual. Allow the vial to stand undisturbed for approximately 5 minutes. The solution should be essentially free of visible particulates, clear to slightly opalescent and colorless to pale yellow.

Determine the number of mg of Trastuzumab needed, based on a loading dose of 4 mg Trastuzumab/kg body weight or a maintenance dose of 2 mg Trastuzumab/kg body weight. Calculate the volume of 21 mg/mL Trastuzumab solution and withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% Sodium Chloride Injection, USP. **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED.** Gently invert the bag to mix the solution. The reconstituted preparation results in a colorless to pale yellow transparent solution. Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.

No incompatibilities between HERCEPTIN and polyvinylchloride or polyethylene bags have been observed.

Administration
Treatment may be administered in an outpatient setting by administration of a 4 mg/kg Trastuzumab loading dose by intravenous (IV) infusion over 90 minutes. **DO NOT ADMINISTER AS AN IV PUSH OR BOLUS.** Patients should be observed for fever and chills or other infusion-associated symptoms (see BOXED WARNINGS, WARNINGS, and ADVERSE REACTIONS). If prior infusions are well tolerated, subsequent weekly doses of 2 mg/kg Trastuzumab may be administered over 30 minutes.

HERCEPTIN should not be mixed or diluted with other drugs. HERCEPTIN infusions should not be administered or mixed with dextrose solutions.

Stability and Storage
Vials of HERCEPTIN are stable at 2–8°C (36–46°F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of HERCEPTIN reconstituted with BWFI, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2–8°C (36–46°F), and the solution is preserved for multiple use. Discard any remaining multi-dose reconstituted solution after 28 days. If unpreserved SWFI (not supplied) is used, the reconstituted HERCEPTIN solution should be used immediately and any unused portion must be discarded. DO NOT FREEZE HERCEPTIN THAT HAS BEEN RECONSTITUTED.

The solution of HERCEPTIN for infusion diluted in polyvinylchloride or polyethylene bags containing 0.9% Sodium Chloride Injection, USP, may be stored at 2–8°C (36–46°F) for up to 24 hours prior to use. Diluted HERCEPTIN has been shown to be stable for up to 24 hours at room temperature (2–25°C). However, because diluted HERCEPTIN contains no effective preservative, the reconstituted and diluted solution should be stored refrigerated (2–8°C).

How Supplied
HERCEPTIN (Trastuzumab) is supplied as a lyophilized, sterile powder nominally containing 440 mg Trastuzumab per vial under vacuum.

Each carton contains one vial of 440 mg HERCEPTIN® (Trastuzumab) and one vial containing 20 mL of Bacteriostatic Water for Injection, USP, 1.1% benzyl alcohol. NDC 50242-134-68.

REFERENCES

1. Coussens L, Yang-Feng TL, Liao Y-C, Chen E, Gray A, McGrath J, et al. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science.* 1985;230:1132-1139.
2. Press MF, Pike MC, Chazin VR, Hung G, Udove JA, Markowicz M, et al. Her-2/*neu* expression in node-negative breast cancer: direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. *Cancer Res.* 1993;53:4960-4970.
3. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. *Science.* 1989;244:707-712.
4. Kallioniemi O-P, Kallioniemi A, Kurisu W, Thor A, Chen L-C, Smith HS, et al. *ERBB2* amplification in breast cancer analyzed by fluorescence in situ hybridization. *Proc Natl Acad Sci USA.* 1992;89:5321-5325.
5. Paulletti G, Godolphin W, Press MF, Slamon DJ. Detection and quantitation of HER-2/*neu* gene amplification in human breast cancer archival material using fluorescence in situ hybridization. *Oncogene.* 1996;13:63-72.
6. Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A. p185^{HER2} monoclonal antibody has antiproliferative effects *in vitro* and sensitizes human breast tumor cells to tumor necrosis factor. *Mol Cell Biol.* 1989;9:1165-1172.
7. Lewis GD, Figari I, Fendly B, Wong WL, Carter P, Gorman C, et al. Differential responses of human tumor cell lines to anti-p185^{HER2} monoclonal antibodies. *Cancer Immunol Immunother.* 1993;37:255-263.
8. Baselga J, Norton L, Albanell J, Kim Y-M, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin®) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/*neu* overexpressing human breast cancer xenografts. *Cancer Res.* 1998;58:2825-2831.
9. Hotaling TE, Reitz B, Wolfgang-Kimball D, Bauer K, Fox JA. The humanized anti-HER2 antibody rhuMAb HER2 mediates antibody dependent cell-mediated cytotoxicity via FcγR III [abstract]. *Proc Am Assoc Cancer Res.* 1996;37:471. Abstract 3215.
10. Pegram MD, Baly D, Wirth C, Gilkerson E, Slamon DJ, Sliwkowski MX, et al. Antibody dependent cell-mediated cytotoxicity in breast cancer patients in Phase III clinical trials of a humanized anti-HER2 antibody [abstract]. *Proc Am Assoc Cancer Res.* 1997;38:602. Abstract 4044.
11. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med.* 2001;344:783-792.
12. Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. Requirement for neuregulin receptor erb82 in neural and cardiac development. *Nature.* 1995;378:394-398.

HERCEPTIN®
(Trastuzumab)
Manufactured by:
Genentech, Inc.
1 DNA Way

LK0726
7172907
4817408
FDA Approval Date February 2005
Code revision February 2005

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

Our Reference No.: 98-0369

September 25, 1998

Robert L. Garnick, Ph.D.
Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Garnick:

Your biologics license application for Trastuzumab is approved effective this date. Genentech, Inc., South San Francisco, California, is hereby authorized to introduce or deliver for introduction into interstate commerce Trastuzumab under Department of Health and Human Services U.S. License No. 1048.

Trastuzumab is indicated for treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have received one or more chemotherapy regimens for their metastatic disease. Trastuzumab in combination with paclitaxel is indicated for treatment of patients with metastatic breast cancer whose tumors overexpress HER2 protein and who have not received chemotherapy for their metastatic disease. In accordance with approved labeling, your product will bear the tradename Herceptin and will be marketed in 440 mg multi-dose vials supplied with Bacteriostatic Water for Injection, USP (containing 1.1% benzyl alcohol).

You are not currently required to submit samples of future lots of Trastuzumab to the Center for Biologics Evaluation and Research (CBER) for release by the Director, CBER, under 21 CFR 610.2. FDA will continue to monitor compliance with 21 CFR 610.1 requiring assay and release of only those lots that meet release specifications.

The dating period for this product shall be 30 months from the date of manufacture when stored at 2-8°C. The date of manufacture shall be defined as the date of final sterile filtration of the final formulated product. The bulk antibody may be stored for up to 24 months at -20°C. The dating period for the diluent, Bacteriostatic Water for Injection shall be 24 months. The expiration date for the packaged product, Herceptin plus diluent, shall be dependent on the shortest expiration date of either component. Results of stability studies from the first three production lots should be submitted throughout the dating period on an annual basis.

Any changes in the manufacture, packaging or labeling of the product or in the manufacturing facilities will require the submission of information to your biologics license application for our review and written approval consistent with 21 CFR 601.12.

We acknowledge your written commitments of August 7, 1998 and September 22, 1998, and as agreed during discussions on September 25, 1998, which include the following:

1. Within one year of approval, the stability of the reconstituted product stored under the "worst case" conditions will be studied. Results including IEC and the antiproliferative assay will be submitted for review.

2. For the next five lots to be released, the antiproliferative assay will be performed using three replicates of three dilutions, however, the lots will be released as per the actual SOP(Q12333). These additional test results will be submitted for review.

3. To develop and conduct a clinical trial which addresses the impact on progression-free survival and response rate of the addition of Herceptin therapy to chemotherapy as compared with chemotherapy alone in patients with 2+ HER2 (weakly positive) overexpression.

4. To obtain ejection fraction data at baseline and at scheduled periodic monitoring intervals in the following Herceptin breast cancer clinical trials:

    - Carboplatin-Paclitaxel-Herceptin vs Paclitaxel-Herceptin (total n ≅ 200)
    - Weekly Paclitaxel-Herceptin (total n ≡ 100)
    - and selected other large clinical trials

5. To assess the ability of medical history, physical exam, and baseline and on-study monitoring of cardiac function to predict and diminish the risk of Herceptin-induced cardiotoxicity. In patients with early signs of Herceptin-induced cardiotoxicity:

    - To evaluate the advisability of discontinuation of Herceptin
    - To evaluate the safety of continuation or reinstitution of Herceptin therapy.

6. To investigate further the safety and efficacy of Herceptin and the risk factors for cardiotoxicity and adequacy of monitoring for cardiotoxicity in the following settings:

    - In a population who has recently received anthracyclines (e.g., collaborative group adjuvant study of AC x 4 followed by Paclitaxel-Herceptin or Paclitaxel alone x 4) and/or in a population in which Herceptin is administered concurrently with anthracycline therapy (e.g., NCI-sponsored study of Herceptin + Doxil® or Herceptin + prolonged infusion of doxorubicin)

    - In a population not previously treated with anthracyclines (e.g., possible collaborative group adjuvant study of taxane/Herceptin regimen in anthracycline naïve patients)

7. To assess the clinical outcome of patients selected for treatment on the basis of the DAKO test and other HER2 diagnostics in the context of Herceptin clinical trials.

8. To perform formal pharmacokinetic interaction studies by assessing serum concentrations of antibody and of drug in human studies of Herceptin + antineoplastic agents (e.g., paclitaxel, doxorubicin).

9. To evaluate the use of Herceptin with antimetabolites in a breast cancer clinical trial of cyclophosphamide, methotrexate, and 5-fluorouracil ± Herceptin.

It is requested that adverse experience reports be submitted in accordance with the adverse experience reporting requirements for licensed biological products (21 CFR 600.80) and that distribution reports be submitted as described (21 CFR 600.81). All adverse experience reports should be prominently identified according to 21 CFR 600.80 and be submitted to the Center for Biologics Evaluation and Research, HFM-210, Food and Drug Administration, 1401 Rockville Pike, Rockville, MD 20852-1448.

Please submit three copies of all final printed labeling at the time of use and include part II of the label transmittal form (FDA Form 2567) with completed implementation information. In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with an FDA Form 2567 or Form 2253 to the Center for Biologics Evaluation and Research, Advertising and Promotional Labeling Staff, HFM-202, 1401 Rockville Pike, Rockville, MD 20852-1448. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by an FDA Form 2567 or Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. No comparative promotional claim or claim of superiority over other similar products should be made unless data to support such claims are submitted to and approved by the Center for Biologics Evaluation and Research.

Sincerely yours,

Jay P. Siegel, M.D., FACP
Director
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

SYNAGIS®
(PALIVIZUMAB)

for Intramuscular Administration

DESCRIPTION: Synagis® (palivizumab) is a humanized monoclonal antibody (IgG1κ) produced by recombinant DNA technology, directed to an epitope in the A antigenic site of the F protein of respiratory syncytial virus (RSV). Palivizumab is a composite of human (95%) and murine (5%) antibody sequences. The human heavy chain sequence was derived from the constant domains of human IgG1 and the variable framework regions of the V_H gene, Cor (1) and Cess (2). The human light chain sequence was derived from the constant domain of C_K and the variable framework regions of the V_K gene, KI01 with Jκ-4 (3). The murine sequences were derived from a murine monoclonal antibody, Mab 1129 (4), by a process which involves the grafting of the murine complementarity determining regions into the human antibody frameworks. Synagis® is composed of two heavy chains and two light chains and has a molecular weight of approximately 148,000 Daltons.

Synagis® is supplied as a sterile lyophilized product for reconstitution with sterile water for injection. Reconstituted Synagis® is to be administered by intramuscular injection only. Upon reconstitution, Synagis® contains the following excipients: 47 mM histidine, 3.0 mM glycine and 5.6% mannitol and the active ingredient, palivizumab, at a concentration of 100 milligrams per ml solution. The reconstituted solution should appear clear or slightly opalescent.

CLINICAL PHARMACOLOGY: *Mechanism of Action:* Synagis® exhibits neutralizing and fusion-inhibitory activity against RSV. These activities inhibit RSV replication in laboratory experiments. Although resistant RSV strains may be isolated in laboratory studies, a panel of 57 clinical RSV isolates were all neutralized by Synagis® (5). Synagis® serum concentrations of ≥40 μg/ml have been shown to reduce pulmonary RSV replication in the cotton rat model of RSV infection by 100-fold (5). The *in vivo* neutralizing activity of the active ingredient in Synagis® was assessed in a randomized, placebo-controlled study of 35 pediatric patients chronically hospitalized because of RSV disease. In these patients, palivizumab significantly reduced the quantity of RSV in the lower respiratory tract compared to control patients (6).

Pharmacokinetics: In studies in adult volunteers Synagis® had a pharmacokinetic profile similar to a human IgG1 antibody in regard to the volume of distribution and the half-life (mean 18 days). In pediatric patients less than 24 months of age, the mean half-life of Synagis® was 20 days and monthly intramuscular doses of 15 mg/kg achieved mean ±SD 30 day trough serum drug concentrations of 37 ±21 μg/mL after the first injection, 57 ±41 μg/mL after the second injection, 68 ±51 μg/mL after the third injection and 72 ±50 μg/mL after the fourth injection (7). In pediatric patients given Synagis® for a second season, the mean ±SD serum concentrations following the first and fourth injections were 61 ±17 μg/mL and 86 ±31 μg/mL, respectively.

CLINICAL STUDIES: The safety and efficacy of Synagis® were assessed in a randomized, double-blind, placebo-controlled trial (IMpact-RSV Trial) of RSV disease prophylaxis among high-risk pediatric patients (7). This trial, conducted at 139 centers in the United States, Canada and the United Kingdom, studied patients ≤24 months of age with bronchopulmonary dysplasia (BPD) and patients with premature birth (≤35 weeks gestation) who were ≤6 months of age at entry entry. Patients with uncorrected congenital heart disease were excluded from enrollment. In this trial, 500 patients were randomized to receive five monthly placebo injections and 1,002 patients were randomized to receive five monthly injections of 15 mg/kg of Synagis®. Subjects were randomized into the study from November 15 to December 13, 1998, and were followed for safety and efficacy for 150 days. Ninety-nine percent of all subjects completed the study and 93% received all five injections. The primary endpoint was the incidence of RSV hospitalization.

RSV hospitalizations occurred among 53 of 500 (10.6%) patients in the placebo group and 48 of 1,002 (4.8%) patients in the Synagis® group, a 55% reduction (p<0.001). The reduction of RSV hospitalization was observed both in patients enrolled with a diagnosis of BPD (34/266 [12.8%] placebo vs 39/496 [7.9%] Synagis®) and patients enrolled with a diagnosis of prematurity without BPD (19/234 [8.1%] placebo vs 9/506 [1.8%] Synagis®). The reduction of RSV hospitalization was observed throughout the course of the RSV season.

Among secondary endpoints, the incidence of ICU admission during hospitalization for RSV infection was lower among subjects receiving Synagis® (1.3%) than among those receiving placebo (3.0%), but there was no difference in the mean duration of ICU care between the two groups for patients requiring ICU care. Overall, the data do not suggest that RSV illness was less severe among patients who received Synagis® and who required hospitalization due to RSV infection than among placebo patients who required hospitalization due to RSV infection. Synagis® did not alter the incidence and mean duration of hospitalization for non-RSV respiratory illness or the incidence of otitis media.

INDICATIONS AND USAGE: Synagis® is indicated for the prevention of serious lower respiratory tract disease caused by respiratory syncytial virus (RSV) in pediatric patients at high risk of RSV disease. Safety and efficacy were established in infants with bronchopulmonary dysplasia (BPD) and infants with a history of prematurity (≤35 weeks gestational age). (See *Clinical Studies* section)

CONTRAINDICATIONS: Synagis® should not be used in pediatric patients with a history of a severe prior reaction to Synagis® or other components of this product.

WARNINGS: Anaphylactoid reactions following the administration of Synagis® have not been observed but can occur following the administration of proteins. If anaphylaxis or severe allergic reaction occurs, administer epinephrine (1:1000) and provide supportive care as required.

PRECAUTIONS: *General:* Synagis® is for intramuscular use only. As with any intramuscular injection, Synagis® should be given with caution to patients with thrombocytopenia or any coagulation disorder.

The safety and efficacy of Synagis® have not been demonstrated for treatment of established RSV disease.

The single-use vial of Synagis® does not contain a preservative. Injections should be given within 6 hours after reconstitution.

Immunogenicity: In the IMpact-RSV trial, the incidence of anti-humanized antibody following the fourth injection was 1.1% in the placebo group and 0.7% in the Synagis® group. In pediatric patients receiving Synagis® for a second season, one of fifty-six patients had transient, low titer reactivity. This reactivity was not associated with adverse events or alterations in Synagis® serum concentrations.

Drug Interactions: No formal drug-drug interaction studies were conducted. In the IMpact-RSV trial, the proportions of patients in the placebo and Synagis® groups who received routine childhood vaccines, influenza vaccine, bronchodilators or corticosteroids were similar and no incremental increase in adverse reactions was observed among patients receiving these agents.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenesis, mutagenesis and reproductive toxicity studies have not been performed.

Pregnancy: Pregnancy Category C: Synagis® is not indicated for adult usage and animal reproduction studies have not been conducted. It is also not known whether Synagis® can cause fetal harm when administered to a pregnant woman or could affect reproductive capacity.

ADVERSE REACTIONS: In the combined pediatric prophylaxis studies of pediatric patients with BPD or prematurity involving 520 subjects receiving placebo and 1160 subjects receiving Synagis® (palivizumab), the proportions of subjects in the placebo and Synagis® groups who experienced any adverse event or any serious adverse event were similar.

Most of the safety information was derived from the IMpact-RSV trial. In this study, Synagis® was discontinued in five patients: two because of vomiting and diarrhea, one because of erythema and moderate induration at the site of the fourth injection, and two because of pre-existing medical conditions which required management (one with congenital anemia and one with pulmonary venous stenosis requiring cardiac surgery). Deaths in study patients occurred in five of 500 placebo recipients and four of 1,002 Synagis® recipients. Sudden infant death syndrome was responsible for two of these deaths in the placebo group and one death in the Synagis® group. Adverse events which occurred in more than 1% of patients receiving Synagis® in the IMpact-RSV study for which the incidence in the Synagis® group was 1% greater than in the placebo group are shown in Table 1.

Table 1. Adverse Events Occurring in IMpact-RSV Study at Greater Frequency in the Synagis® Group

% of patients with:	Placebo n = 500	Synagis® n = 1,002
upper respiratory infection	49.0%	52.6%
otitis media	40.0%	41.2%
rhinitis	33.4%	38.7%
rash	22.4%	25.6%
pain	6.8%	8.3%
hernia	5.0%	6.3%
SGOT increased	3.8%	4.9%
pharyngitis	1.4%	2.6%

Other adverse events reported in more than 1% of the Synagis® group included: fever, cough, wheeze, bronchiolitis, pneumonia, bronchitis, asthma, croup, dyspnea, sinusitis, apnea, failure to thrive, nervousness, diarrhea, vomiting, and gastroenteritis. SGPT increase, liver function abnormality, study drug injection site reaction, conjunctivitis, viral infection, oral moniliasis, fungal dermatitis, eczema, seborrhea, anemia and flu syndrome. The incidence of these adverse events was similar between the Synagis® and placebo groups.

OVERDOSAGE: No data from clinical studies are available on overdosage. No toxicity was observed in infants administered a single intramuscular or subcutaneous injection of Synagis® at a dose of 50 mg/kg. No data are available from human subjects who have received more than 5 monthly Synagis® doses during a single RSV season.

DOSAGE AND ADMINISTRATION: The recommended dose of Synagis® is 15 mg/kg of body weight. Patients, including those who develop an RSV infection, should receive monthly doses throughout the RSV season. The first dose should be administered prior to commencement of the RSV season. In the northern hemisphere, the RSV season typically commences in November and lasts through April, but it may begin earlier or persist later in certain communities.

Synagis® should be administered in a dose of 15 mg/kg intramuscularly using aseptic technique, preferably in the anterolateral aspect of the thigh. The gluteal muscle should not be used routinely as an injection site because of the risk of damage to the sciatic nerve. The dose per month = [patient weight (kg) x 15 mg/kg ÷ 100 mg/mL of Synagis®]. Injection volumes over 1 mL should be given as a divided dose.

Preparation for Administration:

- To reconstitute, remove the tab portion of the vial cap and clean the rubber stopper with 70% ethanol or equivalent.
- Both the 50 mg and 100 mg vials contain an overfill to allow the withdrawal of 50 milligrams or 100 milligrams respectively when reconstituted following the directions described below.
- Slowly add 0.6 mL of sterile water for injection to the 50 mg vial or add 1.0 mL of sterile water for injection to the 100 mg vial. The vial should be gently swirled for 30 seconds to avoid foaming. DO NOT SHAKE VIAL.
- Reconstituted Synagis® should stand at room temperature for a minimum of 20 minutes until the solution clarifies.
- Reconstituted Synagis® does not contain a preservative and should be administered within 6 hours of reconstitution.

To prevent the transmission of hepatitis viruses or other infectious agents from one person to another, sterile disposable syringes and needles should be used. Do not reuse syringes and needles.

HOW SUPPLIED: Synagis® is supplied in single use vials as lyophilized powder to deliver either 50 milligrams or 100 milligrams when reconstituted with sterile water for injection.

50 mg vial NDC 60574-4112-1
Upon reconstitution the 50 mg vial contains 50 milligrams Synagis® in 0.5 mL

100 mg vial NDC 60574-4111-1
Upon reconstitution the 100 mg vial contains 100 milligrams Synagis® in 1.0 mL

Upon receipt and until reconstitution for use, Synagis® should be stored between 2 and 8°C (35.6° and 46.4°F) in its original container. Do not freeze. Do not use beyond the expiration date.

REFERENCES:

1. Press E, and Hogg N. The amino acid sequences of the Fd Fragments of Two Human gamma-1 heavychains. Biochem. J. 1970;117: 641-660.
2. Tajahami N, Norma T, and Hanjo T. Rearranged immunoglobulin heavy chain variable region (Vh) pseudogene that deletes the second complementarity-determining region. Proc. Nat. Acad. Sci. USA1994;81:5194-5198.
3. Bentley D, and Rabbitts T. Human immunoglobulin variable region genes - DNA sequences of two Vk genes and a pseudogene. Nature 1980;288:730-733.
4. Beeler JA, and Van Wyke Coelingh K. Neutralization epitopes of the F Protein of Respiratory Syncytial Virus: Effect of mutation upon fusion function. J Virology 1989;63:2941-2950.
5. Johnson S, Oliver C, Prince GA, et al. Development of a humanized monoclonal antibody (MEDI-493) with potent in vitro and in vivo activity against respiratory syncytial virus. J Infect. Dis. 1997;176:1215-1224.
6. Malley R, DeVincenzo J, Ramilo O, et al. Reduction of Respiratory Syncytial Virus (RSV) in Tracheal Aspirates in Intubated Infants by Use of Humanized Monoclonal Antibody to RSV F Protein. J Infect. Dis. 1998;178:1555-1561.
7. The IMpact RSV Study Group. Palivizumab, a Humanized Respiratory Syncytial Virus Monoclonal Antibody, Reduces Hospitalization From Respiratory Syncytial Virus Infection in High-risk Infants. Pediatrics 1998;102:531-537.

Manufactured by:
MedImmune, Inc.
Gaithersburg, MD 20878
U.S. Gov't. License No. 1252
(1-877-633-4411)

Co-Marketed by:
ROSS PEDIATRIC DIVISION
ABBOTT LABORATORIES INC.
COLUMBUS, OHIO 43216-6234

Rev. date: December 2, 1999